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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte MEHRAN M. KHODADOUST

Appeal 2010-008812
Application 10/029,471
Technology Center 1600

Before DONALD E. ADAMS, DEMETRA J. MILLS, and JEFFREY N. FREDMAN, *Administrative Patent Judges.*

FREDMAN, *Administrative Patent Judge.*

DECISION ON APPEAL[1]

This is an appeal under 35 U.S.C. § 134 involving claims to a nucleic acid comprising an expression cassette. The Examiner rejected the claims as anticipated and obvious.

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[1] The two-month time period for filing an appeal or commencing a civil action, as recited in 37 C.F.R. § 1.304, or for filing a request for rehearing, as recited in 37 C.F.R. § 41.52, begins to run from the "MAIL DATE" (paper delivery mode) or the "NOTIFICATION DATE" (electronic delivery mode) shown on the PTOL-90A cover letter attached to this decision.

We have jurisdiction under 35 U.S.C. § 6(b). We reverse and enter a new ground of rejection.

Statement of the Case

The Claims

Claims 83-109 are on appeal. Claims 83 and 85-87 are representative. Claims 83 and 85-87 read as follows:

> 83. A nucleic acid including:
> (a) a splice acceptor site;
> (b) a cassette including in any order a negative selection marker, a positive selection marker, and a reporter gene, wherein said negative selection marker, said positive selection maker, and said reporter gene are integrated into the genome of at least one cell and responsive to one or more endogenous regulatory elements in said at least one cell after said nucleic acid is contacted with a cell.
>
> 85. The nucleic acid of [claim 83, further comprising an internal ribosome entry site] including in 5' to 3' orientation,
> (a) said splice acceptor site; (b) said negative selection marker and said positive selection marker; (c) said internal ribosome entry site; and (d) said reporter gene; or
> (a) said splice acceptor site; (b) said internal ribosome entry site; and (c) said negative selection marker, said positive selection marker, and said reporter gene, in any order; or
> (a) said splice acceptor site; (b) said reporter gene; (c) said internal ribosome entry site; and (d) said negative selection marker and said positive selection marker; or
> (a) said splice acceptor site; (b) said positive selection marker and said reporter gene, in any

order; (c) said internal ribosome entry site; and(d) said reporter gene; or

(a) said splice acceptor site; (b) said negative selection marker and said reporter gene, in any order; (c) said internal ribosome entry site; and (d) said positive selection marker.

86. The nucleic acid of claim 83, 84, or 85 further comprising a nucleic acid segment encoding a transactivator polypeptide, wherein said nucleic acid segment encoding a transactivator polypeptide is incorporated in said cassette of said nucleic acid molecule.

87. The nucleic acid of claim 83, 84, or 85 further comprising one or more recombinase signal sequences.

The issues

A. The Examiner rejected claims 83, 84, 88-96, 98-104, 107, and 108 under 35 U.S.C. § 102(b) as anticipated by Baetscher[2] (Ans. 4-6).

B. The Examiner rejected claim 85 under 35 U.S.C. § 103(a) as obvious over Baetscher (Ans. 6-8).

C. The Examiner rejected claims 87 and 106 under 35 U.S.C. § 103(a) as obvious over Baetscher and Zambrowicz[3] (Ans. 8-9).

D. The Examiner rejected claims 86, 97-105, and 107-109 under 35 U.S.C. § 103(a) as obvious over Baetscher and Massie[4] (Ans. 9-10).

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[2] Baetscher et al., US 5,922,601, issued Jul. 13, 1999.
[3] Zambrowicz et al., US 6,436,707 B1, issued Aug. 20, 2002.
[4] Massie et al., *Inducible Overexpression of a Toxic Protein by an Adenovirus Vector with a Tetracycline-Regulatable Expression Cassette*, 72 J. VIROLOGY 2289-2296 (1998).

A. 5 U.S.C. § 102(b) over Baetscher

The Examiner finds that "Baetscher et al teach a gene trap nucleic acid construct comprising the following elements in a 5'-to-3' orientation: Splice acceptor---IRES---Neo-HSV-TK (see for example Figure 2)" (Ans. 4). The Examiner finds that since "the Neomycin resistance gene is a positive selection marker and HSV-TK is a negative selection marker the above specific construct teaches the following general formula: Splice acceptor---IRES---positive selection---negative selection" (*id.*).

The Examiner finds that Baetscher inserts the nucleic acid into a retroviral vector which "further comprises selectable or assayable markers, including those useful in 'fluorescence activated cell sorting' (see for example column 8, lines 50-57). Such a reporter can be a 'protein that spontaneously emits light...Green Fluorescent Protein (GFP)'" (*id.* at 5).

Appellant contends that "Baetscher never teaches a construct having a negative selection marker, positive selection marker, and reporter gene under the control of a host cellular promoter. Instead, Baetscher's constructs that include two selection markers and a reporter gene include a promoter element regulating expression of one of the genes" (App. Br. 8).

Appellant contends that "Appellant's claimed constructs require that all three elements (i.e., the positive and negative selectable markers and the reporter gene) be under the control of an endogenous regulatory element in the host cell" (*id.* at 9).

The issue with respect to this rejection is: Does the evidence of record support the Examiner's conclusion that the Baetscher teaches a nucleic acid comprising all of the required elements of claim 83 as properly interpreted?

Findings of Fact

1. The Specification teaches in some "embodiments, the reporter gene is not operably linked to a promoter in the nucleic acid. In this embodiment, the nucleic acid may be inserted in a cell such that the reporter gene is operably linked to an endogenous promoter" (Spec. 23, ll. 11-14).

2. The Specification teaches that in

> any of the above aspects, a nucleic acid segment encoding a single protein that has both positive selection traits and negative selection traits may be used as the positive and negative selection markers. In other embodiments, the negative selection marker and the positive selection marker encode different proteins. In still other embodiments, the reporter gene is different from the positive selection marker and/or the negative selection marker.

(Spec. 27, ll. 24-29.)

3. Baetscher teaches a nucleic acid construct which "comprises in downstream sequence (i) a cassette having a functional splice acceptor, a translational stop sequence and an internal ribosome entry site and (ii) a promoterless protein coding sequence encoding at least one polypeptide providing positive and negative selection traits" (Baetscher, col. 4, ll. 55-60).

4. Baetscher teaches that the "term 'selectable markers' refers to a variety of gene products to which cells transformed with a retroviral construct can be selected or screened, including drug-resistance markers, antigenic markers useful in fluorescence-activated cell sorting" (Baetscher, col. 8, ll. 51-55).

5. Baetscher teaches that "a protein that spontaneously emits light and can serve as reporter as well as a positive/negative selectable marker in

FACS analysis, is the Green Fluorescent Protein" (Baetscher, col. 10, ll. 15-18).

6. Baetscher teaches that the

> vector is designed to deliver to cells a molecular tag (or trap) which includes one or several drug selectable markers. Upon integration of the molecular tag into chromosomal DNA, the activity of the endogenous gene at the locus of integration can be monitored using drug selection and, upon stimulation of the cells, genetic loci whose activity-shifts result in altered levels of steady state MRNA, can be molecularly identified and the corresponding genes isolated and characterized.

(Baetscher, col. 13, ll. 25-33.)

Principles of Law

"[A]nticipation of a claim under § 102 can be found only if the prior art reference discloses every element of the claim ...." *In re King*, 801 F.2d 1324, 1326 (Fed. Cir. 1986) (citing *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1457 (Fed. Cir. 1984)). "[A]bsence from the reference of any claimed element negates anticipation." *Kloster Speedsteel AB v. Crucible, Inc.*, 793 F.2d 1565, 1571 (Fed. Cir. 1986).

Analysis

The Examiner contends that "Appellants do not require that the reporter gene be promoterless, only that once it is integrated, it is responsive to an endogenous promoter" (Ans. 11). While this is correct insofar as it goes, when the Examiner argues that upon integration, an "exogenous" promoter becomes "endogenous," this interpretation is simply unreasonable in light of the claim and specification (*see* FF 1). Equally unreasonable is

the Examiner's argument that since any polypeptide can function as a reporter, the "reporter could already be present (integrated) in the cell's genome" (Ans. 12). Claim 83 requires that the responsiveness to endogenous regulatory elements occur after the nucleic acid comes in contact with and integrates into a cell, which reasonably excludes proteins already found within the cell.

Consequently, while Baetscher teaches a nucleic acid with a splice acceptor site, a negative selection marker, and a positive selection marker which are responsive to endogenous regulatory elements, Baetscher does not teach a reporter in the nucleic acid which is reasonably interpreted as responsive to an "endogenous" regulatory element.

Conclusion of Law

The evidence of record does not support the Examiner's conclusion that the Baetscher teaches a nucleic acid comprising all of the required elements of claim 83 as properly interpreted.

B. C., and D. 35 U.S.C. § 103(a)

For the reasons set forth above Baetscher, as it is relied upon by the Examiner, fails to suggest Appellants' claimed invention. Zambrowicz and Massie fail to make up for the deficiencies in Baetscher. Accordingly, we reverse the obviousness rejections based on Baetscher alone, in combination with either Zambrowicz or Massie.

New Grounds of Rejection

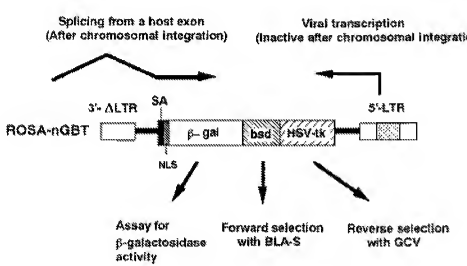
Under the provisions of 37 C.F.R. § 41.50(b), we enter the following new grounds of rejection.

Anticipation- Akiyama

Claims 83, 88 and 90-96 are rejected under 35 U.S.C. § 102(b)[5] as anticipated by Akiyama.[6]

Findings of Fact

7. Akiyama teaches a vector in figure 1 as reproduced below:



"FIG. 1. Gene trap strategy with ROSA-nGBT vector" (Akiyama 3267, col. 2).

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[5] We have reviewed US application 09/697,843, filed October 27, 2000, and do not find descriptive support for the instantly claimed invention. Therefore, Appellants are only entitled to benefit of priority to US Application 09/908,305, filed July 17, 2001.

[6] Akiyama et al., *Identification of a Series of Transforming Growth Factor β-Responsive Genes by Retrovirus-Mediated Gene Trap Screening*, 20 MOLECULAR CELLULAR BIOLOGY 3266-3273 (May 2000) (cited on PTO-1449 filed on May 31, 2002).

8. Akiyama teaches "a gene trap vector carrying a newly developed reporter gene, nGBT, next to the splice acceptor site" (Akiyama 3267, col. 2).

9. Akiyama teaches that the "nGBT gene encodes a fusion protein consisting of four parts: NLS, β-Gal, BLA-S deaminase, and HSV TK . . . a chimeric transcript containing a 5' segment of the host gene (trapped gene) spliced into the splice acceptor sequence located at 5' end of the nGBT reporter gene will be expressed" (Akiyama 3267, col. 2).

10. Akiyama teaches that such "cell clones (trap lines) will become resistant to BLA-S and sensitive to GCV. The expression profile of the trapped gene can be monitored using β-Gal activity" (Akiyama 3267, col. 2).

11. Akiyama teaches that "A549 cells . . . were infected with ROSA-nGBT virus" (Akiyama 3267, col. 1).

Principles of Law

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987).

Analysis

Akiyama teaches a nucleic acid which includes a splice acceptor site (FF 7-8). Akiyama teaches a cassette which comprises a reporter gene, β-Gal; a negative selection marker, HSV-TK; and a positive selection marker, BLA-S deaminase (FF 7, 9). Akiyama teaches that after integration of the vector into a cell, the β-gal, BLA-S deaminase, and HSV TK will become responsive to the "endogenous" promoter, where the "endogenous" promoter

is a promoter found in the cell, not in the nucleic acid being integrated (FF 7, 9, 10).

With regard to claim 88, Akiyama teaches negative selection using HSV-tk (FF 7).

With regard to claims 90 and 91, Akiyama teaches a reporter which is the enzyme β-galactosidase which is operably linked to a host cellular gene after integration of the vector into a cell (FF 9).

With regard to claim 92, Akiyama teaches that the nucleic acid is in a vector (FF 8).

With regard to claims 93 and 94, Akiyama teaches a retroviral vector with an integration sequence (FF 11).

With regard to claim 95, Akiyama teaches integration of the vector into a cell (FF 10).

With regard to claim 96, Akiyama teaches that the cell will be sensitive to GCV (FF 10).

Obviousness – Akiyama and Baetscher

Claims 84, 85 and 89 are rejected under 35 U.S.C. § 103(a) as obvious over Akiyama and Baetscher.

Findings of Fact

12. Baetscher teaches that an "'internal ribosome entry site'(IRES) is an element which permits attachment of a downstream coding region or open reading frame with a cytoplasmic polysomal ribosome for purposes of initiating translation thereof in the absence of any internal promoters. An

IRES is included to initiate translation of selectable marker protein coding sequences" (Baetscher, col. 8, ll. 10-16).

13. Baetscher teaches that "[e]xpression of such positive selectable marker genes is made detectable by supplementing the culture medium with the corresponding drug, G418, hygromycin and puromycin, respectively" (Baetscher, col. 9, ll. 61-64).

Principles of Law

The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the claimed invention and the prior art; and (4) secondary considerations of nonobviousness, if any. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). The Supreme Court has recently emphasized that "the [obviousness] analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007).

Analysis

Akiyama teaches a cassette as discussed above (FF 7-11). Akiyama does not teach an internal ribosome entry site or different positive selection markers.

Baetscher teaches the use of an internal ribosome entry site (FF 12) as well as a variety of different positive selection markers (FF 13).

It would have been prima facie obvious to one of ordinary skill at the time the invention was made to incorporate the internal ribosome entry site

and positive selection markers of Baetscher into the vector of Akiyama since Baetscher teaches that incorporation of the internal ribosome entry site enhances the initation of translation of selection and marker proteins (FF 12) and since Baetscher teaches the efficacy of the alternative selectable markers. An ordinary artisan would have recognized that the order of elements in the vector is routinely optimizable. Such a combination is merely a "predictable use of prior art elements according to their established functions." *KSR*, 550 U.S. at 417.

Obviousness – Akiyama, Baetscher, and Tessier-Lavigne

Claims 86, 97-105 and 107-109 are rejected under 35 U.S.C. § 103(a) as obvious over Akiyama, Baetscher, and Tessier-Lavigne.[7]

Findings of Fact

14. The Specification teaches that a "'transactivator' and a 'transactivator polypeptide' are nucleic acid sequences and polypeptides, respectively, that transcribes, or causes the transcription of, a protein which effects the regulation of a genomic loci. Examples of transactivator polypeptides include transcription factors and growth factors" (Spec. 34, ll. 16-19).

15. Tessier-Lavigne teaches "gene trap vectors comprise a polynucleotide comprising promoterless selectable marker and axon reporter encoding sequences, whereupon transfer into a vertebrate . . . the cell

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[7] Tessier-Lavigne et al., US 6,248,934 B1, issued Jun. 19, 2001 (cited on PTO-892, mailed Dec. 23, 2004).

expresses the selectable marker and the axon reporter under the transcriptional control of the gene" (Tessier-Lavigne, col. 3, ll. 60-67).

16. Tessier-Lavigne teaches that "[s]pecific expression of the targeted gene product is achieved using a binary system. In this case the gene trap vectors comprise a polynucleotide comprising promoterless selectable marker and transcription factor encoding sequences, optionally operatively joined to a functional internal ribosome-entry site (IRES)" (Tessier-Lavigne, col. 4, ll. 35-40).

17. Tessier-Lavigne teaches that a

> wide variety of transcription factors can be used. Factors to include in selecting a suitable transcription factor include high-level and specific expression from the operator sequence in vertebrate cells, lack of cytotoxic or other phenotypic effects in vertebrate cells due to expression of the transcription factor alone, and sensitivity of the transcription factor to drugs, hormones, or other compounds which can be used to temporally control expression.

(Tessier-Lavigne, col. 4, ll. 49-57.)

18. Tessier-Lavigne teaches that "[e]xemplary transcription factors include . . . the tet transactivator, tTA" (Tessier-Lavigne, col. 4, ll. 57-59).

19. Tessier-Lavigne teaches that this "system allows amplification of the axon reporter signal as well as creating a more versatile insertion which can be used to drive expression of any desired targeted gene under the operative control of the transcription factor" (Tessier-Lavigne, col. 3, ll. 11-14).

Analysis

Akiyama and Baetscher teach cassettes as discussed above (FF 7-13). Akiyama and Baetscher do not teach the use of a transactivator polypeptide.

Tessier-Lavigne teaches incorporation of sequences encoding transcription factors (transactivator polypeptides as per the Specification (FF 14)) into promoter trap vectors (FF 15-18).

With regard to claims 86, 97, 98, 107-109, Tessier-Lavigne specifically teaches "gene trap vectors comprise a polynucleotide comprising promoterless selectable marker and transcription factor encoding sequences, optionally operatively joined to a functional internal ribosome-entry site (IRES)" (Tessier-Lavigne, col. 4, ll. 37-40).

With regard to claim 99, Akiyama teaches negative selection using HSV-tk (FF 7).

With regard to claim 100, Baetscher teaches that "[e]xpression of such positive selectable marker genes is made detectable by supplementing the culture medium with the corresponding drug, G418, hygromycin and puromycin, respectively" (Baetscher, col. 9, ll. 61-64; FF 13).

With regard to claims 101-104, Akiyama teaches a reporter which is the enzyme β-galactosidase which is operably linked to a host cellular gene after integration of the vector into a cell (FF 9).

With regard to claim 105, Tessier-Lavigne teaches that "[e]xemplary transcription factors include . . . the tet transactivator, tTA" (Tessier-Lavigne, col. 4, ll. 57-59; FF 18).

It would have been prima facie obvious to one of ordinary skill at the time the invention was made to incorporate transactivator polypeptides of

Tessier-Lavigne with the gene trap vectors of Akiyama and Baetscher since Tessier-Lavigne teaches that this "system allows amplification of the axon reporter signal as well as creating a more versatile insertion which can be used to drive expression of any desired targeted gene under the operative control of the transcription factor" (Tessier-Lavigne, col. 3, ll. 11-14; FF 19). An ordinary artisan would have modified the gene trap vectors of Akiyama and Baetscher with the incorporation of a transcription factor as taught in the gene trap vector of Tessier-Lavigne in order to permit a more versatile insertion, where necessary, as suggested by Tessier-Lavigne (FF 19). Such a combination is merely a "predictable use of prior art elements according to their established functions." *KSR*, 550 U.S. at 417.

Obviousness – Akiyama, Baetscher, Tessier-Lavigne, and Zambrowicz

Claims 87 and 106 are rejected under 35 U.S.C. § 103(a) as obvious over Akiyama, Baetscher, Tessier-Lavigne, and Zambrowicz.

Findings of Fact

20. Zambrowicz teaches that:

> Optionally, the 5' gene trap cassette can be flanked by suitable recombinase sites (e.g., lox P, frt, etc.). In one such embodiment, a recombinase site flanked 5' gene trap cassette is used in conjunction with a second 5' gene trap cassette (present downstream from the 3' recombinase site) that encodes a detectable marker, a different selectable marker, or an enzymatic marker (such as, but not limited to, green fluorescent protein, beta lactamase, TK, blasticidin, HPRT, etc.), and that is preferably not be flanked by the same recombinase sites the first 5' gene trap cassette. In the event that both of the 5' gene trap cassettes are not expressed at acceptable levels (via alternative splicing), the

> second 5' gene trap cassette (that encodes a detectable marker) can be "activated" by using a suitable recombinase activity (i.e., cre, flp, etc.) in vitro or in vivo to remove the first (recombinase site flanked) 5' gene trap cassette.

(Zambrowicz, col. 8, ll. 40-55.)

21. Zambrowicz teaches that with "lox sites in the LTRs, once an insertion is made and identified, the cre recombinase, for example, can be added and used to remove the entire insert except for one LTR containing a single frt or lox site. Additionally, a DNA response element that allows regulatable gene expression can be incorporated, wholly or in part, in conjunction with the recombinase sites" (Zambrowicz, col. 27, ll. 36-42).

22. Zambrowicz teaches that

> the flp recombinase, for example, can mediate the replacement of frt flanked integrated vector sequences with exogenously added frt flanked sequences. Accordingly, once a suitably constructed vector (incorporating flanking recombinase sites) is incorporated into a given region of the target cell genome, virtually any of a wide variety of DNA sequences (i.e., promoters, enhancers, IRES, response elements, etc.) that also incorporate the same flanking recombinase sites can be exchanged into or out of the vector by employing the proper recombinase protein.

(Zambrowicz, col. 27, ll. 58-67.)

Analysis

Akiyama, Baetscher, and Tessier-Lavigne teach cassettes as discussed above (FF 7-20). Akiyama, Baetscher, and Tessier-Lavigne do not teach the use of a recombinase sites.

Zambrowicz teaches incorporation of recombinase sites into gene trap cassettes (FF 20).

It would have been prima facie obvious to one of ordinary skill at the time the invention was made to incorporate the recombinase sites of Zambrowicz with the gene trap vectors of Akiyama, Baetscher, and Tessier-Lavigne since Zambrowicz teaches that

> once a suitably constructed vector (incorporating flanking recombinase sites) is incorporated into a given region of the target cell genome, virtually any of a wide variety of DNA sequences (i.e., promoters, enhancers, IRES, response elements, etc.) that also incorporate the same flanking recombinase sites can be exchanged into or out of the vector by employing the proper recombinase protein

(Zambrowicz, col. 27, ll. 58-67; FF 22). An ordinary artisan would have modified the gene trap vectors of Akiyama, Baetscher, and Tessier-Lavigne with the incorporation of a recombinase site as taught in the gene trap vector of Zambrowicz in order to permit a simplified exchange of DNA sequences, as suggested by Zambrowicz (FF 20-22). Such a combination is merely a "predictable use of prior art elements according to their established functions." *KSR*, 550 U.S. at 417.

SUMMARY

In summary, we reverse the rejection of claims 83, 84, 88-96, 98-104, 107, and 108 under 35 U.S.C. § 102(b) as anticipated by Baetscher.

We reverse the rejection of claim 85 under 35 U.S.C. § 103(a) as obvious over Baetscher.

We reverse the rejection of claims 87 and 106 under 35 U.S.C. § 103(a) as obvious over Baetscher and Zambrowicz.

We reverse the rejection of claims 86, 97-105, and 107-109 under 35 U.S.C. § 103(a) as obvious over Baetscher and Massie.

This decision also contains new grounds of rejection pursuant to 37 C.F.R. § 41.50(b) (effective September 13, 2004, 69 Fed. Reg. 49960 (August 12, 2004), 1286 Off. Gaz. Pat. Office 21 (September 7, 2004)). 37 C.F.R. § 41.50(b) provides "[a] new ground of rejection pursuant to this paragraph shall not be considered final for judicial review."

Claims 83-109 are subject to the new grounds of rejection as discussed above.

37 C.F.R. § 41.50(b) also provides that the Appellants, WITHIN TWO MONTHS FROM THE DATE OF THE DECISION, must exercise one of the following two options with respect to the new ground of rejection to avoid termination of the appeal as to the rejected claims:

> (1) Reopen prosecution. Submit an appropriate amendment of the claims so rejected or new evidence relating to the claims so rejected, or both, and have the matter reconsidered by the Examiner, in which event the proceeding will be remanded to the Examiner. . . .
>
> (2) Request rehearing. Request that the proceeding be reheard under § 41.52 by the Board upon the same record. . .
>
> .

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv)(2006).

REVERSED, § 41.50(b)

cdc

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